Invasion by *Hakea sericea* Schrad. in Western Iberia: drivers, patterns and relation with fire – a multiscale spatial modelling framework

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.
O Presidente do Júri,

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Abstract

Invasion by alien species represents a significant threat for ecosystems worldwide, with impacts over both natural and human dimensions, in some cases leading to ecological and socioeconomic regime shifts. Invasive species are also able to modify disturbance regimes, such as fire occurrence and dynamics. Biodiversity hotspots, areas that feature both a large concentration of endemic *taxa* and serious habitat loss, are particularly important areas worldwide for biodiversity conservation. Regions with a Mediterranean-type of climate are known for the high levels of biodiversity and endemism occurrence, but also for their susceptibility to invasions.

Hakea sericea Schrad., a plant species belonging to the *Proteaceae* family and native to Australia, has become a serious invader in two regions of the world, South Africa and Portugal, both biodiversity hotspots with Mediterranean-type of climate. *H. sericea* presents traits related to fire adaptation, and is known to cause changes in fire related variables in invaded areas.

Species Distribution Models (SDMs) have been used extensively in the study of biological invasions, with studies that take into consideration the scale dependent nature of many ecological phenomena being particularly useful. Their ability to forecast potential distributions of invasive species is particularly useful for management, as it allows to direct prevention efforts, regarded as the most cost-effective way of dealing with invasions.

This work presents a multiscale modelling framework, implemented in a regional scale area (Portugal and Galicia), and a nested local scale area in the Minho region in north-western Portugal. A Multimodel Inference Framework (MMI) was used to identify the main drivers of *H. sericea* distribution for each study area and project the potential distribution of the species for the regional scale study area. For the local scale area, an ensemble modelling framework was also used, which allowed to identify the most important predictors of *H. sericea* distribution at the local scale, and to project the potential distribution of the species. This projection was then used to analyse the spatial relation between *H. sericea* distribution and fire occurrence.

Our results indicate that the main set of drivers of the distribution of *H. sericea* at the regional scale are related to climate, followed by landscape composition, and that this ranking is reversed at the local scale. However, the main single driver of the species' distribution in the local scale area was a geological predictor, related to the abundance of schist lithology. Finally, presence of *H. sericea* was shown to be significantly related to fire occurrence in Minho. These results show a scale dependence in the importance of different drivers of invasion, and the importance of geology for the local scale distribution of the species, hypothesized to be caused by specific nutrient absorption adaptations. The relation between

Hakea sericea and fire occurrence and the species' fire adaptations indicate that a shift in fire regime may be occurring in invaded areas.

These results are particularly important for landscape management in Mediterranean regions, where fire is an important disturbance. Preventing or managing landscape invasion by *Hakea* could play an important role in fire prevention and risk management, especially in heavily invaded regions. This would in turn contribute to a more effective control of *Hakea* in invaded regions. Finally, our spatial projections and the scale-dependence of key determinants of *Hakea* invasion support the idea that trans-border action must be considered when effectively dealing with this and other invasive species.

Keywords: Species Distribution Modelling; *Hakea*; Regime shift; Drivers of biological invasions

Resumo

A invasão por espécies exóticas representa uma ameaça significativa para os ecossistemas, com impactos sobre dimensões naturais e humanas, podendo nalguns casos levar a alterações de regimes ecológicos e socioeconómicos. Estas espécies invasoras têm também a capacidade de alterar regimes de perturbação, como o regime de fogos. *Hotspots* de biodiversidade, entendidos como áreas que possuem uma grande concentração de endemismos e que, ao mesmo tempo, sofrem sérias perdas de habitat, são áreas particularmente importantes para a conservação da biodiversidade a nível mundial. As regiões com um clima de tipo mediterrânico, reconhecidas pelos seus elevados níveis de biodiversidade e endemismos, são também reconhecidas pela sua elevada suscetibilidade à invasão.

Hakea sericea Schrad. é uma planta da família *Proteaceae*, nativa da austrália e que se tornou uma séria invasora em duas regiões, África do Sul e Portugal, que incluem áreas de hotspot de biodiversidade com clima de tipo mediterrânico. Esta espécie possui características relacionadas com a adaptação ao fogo, e sabe-se que causa alterações em variáveis relacionadas com o fogo em áreas invadidas.

Modelos de Distribuição de Espécies (Species Distribution Models, SDMs) têm sido usados extensivamente no estudo das invasões biológicas. Nestes, os estudos que têm em consideração a influência da escala em muitos fenómenos ecológicos revelam-se particularmente úteis. A capacidade dos SDMs para prever distribuições potenciais de espécies invasoras é particularmente útil na gestão, uma vez que permite direcionar esforços de prevenção, reconhecidos como a abordagem de gestão de invasões com melhor relação custo-benefício.

Este trabalho apresenta um quadro de modelação multiescalar, implementado numa área de escala regional composta por Portugal e Galiza, e uma área aninhada de escala local inserida na região do Minho, no noroeste de Portugal. Um quadro de inferência multimodelo (Multimodel Inference Framework, MMI) foi usado para identificar os principais determinantes da distribuição de *H. sericea* para cada uma das áreas de estudo, e para projetar a distribuição potencial da espécie para área de estudo de escala regional. Para a área de estudo local, foi também utilizado um quadro de modelação de consenso, que permitiu identificar os preditores mais importantes para a distribuição de *H. sericea* na escala local, e projetar a distribuição potencial da espécie. Esta projeção foi usada para analisar a relação espacial entre a distribuição de *H. sericea* e a ocorrência de fogo.

Os nossos resultados indicam que o principal conjunto de determinantes da distribuição de *H. sericea* à escala regional está relacionado com o clima, seguido pela composição da paisagem, e que esta ordem é invertida na escala local. No entanto, o principal

determinante da distribuição da espécie na área de escala local foi um preditor geológico, relacionado com a abundância de xistos. Finalmente, mostrou-se que a presença de *Hakea sericea* está significativamente relacionada com a ocorrência de fogos, no Minho. Estes resultados demonstram a existência de uma dependência da escala na importância de diferentes determinantes de invasão, bem como a importância da geologia para a distribuição da espécie na escala local, possivelmente causada por adaptações específicas para absorção de nutrientes. A relação entre a presença de *H. sericea* e a ocorrência de fogos, e as adaptações ao fogo da espécie, indicam que uma alteração no regime de fogos pode estar a ocorrer nas áreas invadidas.

Estes resultados são particularmente importantes para a gestão da paisagem em regiões mediterrânicas, onde o fogo é uma perturbação importante. Prevenir ou gerir a invasão da paisagem por *Hakea* pode ter um papel importante na prevenção do fogo e gestão do risco, especialmente nas áreas fortemente invadidas. Por sua vez, isto contribuiria para um controlo mais eficaz de *Hakea* nas regiões invadidas. Finalmente, as nossas projeções espaciais e a dependência da escala em determinantes chave da invasão por *Hakea* apoiam a ideia de que ações transfronteiriças devem ser consideradas para lidar eficazmente com esta e outras espécies invasoras.

Palavras-chave: Modelos de Distribuição de Espécies; *Hakea*; Alterações de regime; Determinantes de invasões biológicas

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AIC – Akaike Information Criterion

ANN – Artificial Neural Network

AnnPrec – Annual precipitation

Ca – Calcium

CTA – Classification Tree Analysis

Δi – AIC difference

DetSedD – Detritic sedimentary rocks and superficial sedimentary deposits (percentage)

f. sp. – *forma specialis*

FDA – Flexible Discriminant Analysis

GAM – Generalized Additive Model

GBM – Generalized Boosting Model

geolSDI – Shannon Diversity Index of geological categories

GLM – Generalized Linear Model

ha – hectare

IgnRock – Felsic and intermediate Igneous Rocks (percentage)

K – Potassium

km – Kilometre

MARS – Multivariate Adaptive Regression Splines

MAXENT – Maximum Entropy

MetRock – Foliated metamorphic rocks (percentage)

Mg – Magnesium

MinTemp - Minimum temperature of the coldest month

mm – millimetre

MMI – Multimodel Inference

mPerAreR – Mean Perimeter-to-Area Ratio

mShaInd – Mean Shape Index

N – Nitrogen

NumPatc – Number of patches

P – Phosphorous

Pi – Inorganic Phosphorous

pAgrico – Agriculture cover (percentage)

pArtFor – Artificial forests cover (percentage)

PrecSea – Precipitation seasonality (Coefficient of variation)

pShrubs – Shrubland cover (percentage)

pUrbanA – Urban areas cover (percentage)

RF – Random Forest

ROC-AUC – Area Under the Receiver Operating Characteristic curve

SDM – Species Distribution Model

shDiInd – Shannon Diversity Index of Land Cover classes

SRE – Surface Range Envelope

TempRan – Temperature annual range

TSS – True Skill Statistic

w_i – Akaike weight

1. Introduction

Invasion by exotic species (see Appendix I for concepts and definitions related to biological invasions) represents a significant threat to ecosystems worldwide, with impacts over both natural and human dimensions, from ecosystem structure and functioning (Vilà et al., 2011) to economy (Pimentel et al., 2005; Vilà et al., 2010) and ecosystem services provision (Vilà et al., 2010). The synergistic interactions between biological invasions, climate and land use changes are expected to cause biotic homogenization, a situation of particular concern in biodiversity hotspots due to their high concentration of endemisms (Thuiller, 2007). Biodiversity hotspots can be defined as areas that have exceptional concentrations of endemic species (with the minimum criteria being 0.5% of the worlds' plant species), and that are at the same time suffering exceptional habitat loss, qualified by the loss of at least 70% of the primary vegetation (Myers et al., 2000). These areas are estimated to contain 44% of all plant species, confined to 1.4% of Earth's land surface (Myers et al., 2000). This concentration of biodiversity makes hotspots prime targets for conservation. Regions around the world with Mediterranean-type of climate are known to comprise high levels of biodiversity and endemicity, and are included in several biodiversity hotspots (Myers et al., 2000). Thus, it is of high importance to early detect or even anticipate new invasions in Mediterranean regions, particularly in Europe, since vulnerability to invasion has been increased by its long-term human occupation and the related shifts in natural disturbance regimes (Brunel et al., 2010). Two disturbances recognized as particularly important in Mediterranean regions are fire (Pausas et al., 2008) and invasion by exotic species (Gaertner et al., 2009; Brunel et al., 2010).

In some cases the changes in the ecosystems receiving invasive species are so massive that result in regime shifts, i.e. changes in ecosystem structure and function by altering the system's internal feedback mechanisms (Gaertner et al., 2014). Invasive species are also known to cause impacts over disturbance regimes (Mack & D'Antonio, 1998). For example, invasive species presence might lead to modifications in fire regimes, by causing changes in fuel properties such as the moisture content of plant tissues, fuel loads, and fuel continuity and packing ratios (Brooks et al., 2004).

The invasion process involves several stages (transport, introduction, establishment and spread), with a series of barriers (e.g. geographical, survival, spread) that must be overcome by a species/population in order to reach, survive and spread in a new non-native territory (Richardson et al., 2000; Blackburn et al., 2011). The success of invasion processes depends on invasiveness, i.e. the features of an organism defining its ability to invade, and on invasibility, i.e. the characteristics of a system that determine its susceptibility to invasion (Richardson et al., 2011). Moreover, the processes and patterns of invasion exhibit spatial variability, and therefore attempts to understand them must consider scale issues (Pauchard

& Shea, 2006; Theoharides & Dukes, 2007). Modelling frameworks where environmental variables are incorporated in models according to their spatial scale of variation produce more informative predictions of invasion than traditional models (Vicente et al., 2011).

Species Distribution Models (SDMs, appendix II), i.e. models that relate the known distribution of a species with the environmental conditions, can help understanding and predicting the potential distribution of a species in a given landscape (Elith & Leathwick, 2009). In invasion ecology, SDMs have been used to test shifts in ecological niches (Petitpierre et al., 2012), to identify the environmental factors driving invasibility of landscapes (Vicente et al., 2010), and to predict potential conflicts between invasive and rare native species (Vicente et al., 2011), among various other applications. From a management perspective, the ability to predict the distribution of invasive species can be very useful, as predictions may be applied to direct prevention efforts, being more cost-effective than control or eradication measures (Davies & Sheley, 2007).

Hakea sericea Schrad., a woody plant species of the *Proteaceae* family native to South-eastern Australia, is an example of a novel invader in Europe (see appendix III for a detailed overview of the species). This fire-prone shrub has been reported as invasive in South Africa (appendix IIIb) (van Wilgen & Richardson, 1985; Richardson et al., 1987) and more recently in Europe, more precisely in Portugal (appendix IIIc) (Almeida & Freitas, 2006). Early observations indicate that invasion of the South African fynbos biome (itself a fire-adapted system) led to changes in various fire related variables, such as an increase in fuel loads and surface area to volume ratio, and a decrease in live fuel moisture content. While simulations indicated that invasion may decrease the ratio of fire spread, due to a more densely packed fuel bed in comparison with natural fynbos, it is expected that the situation would be inverted in extreme weather conditions, with an increase in fire intensity due to the larger fuel loads (van Wilgen & Richardson, 1985). Due to its capacity to sustain and even promote fire disturbances, potentially leading to ecological and socioeconomic regime shifts (Gaertner et al., 2014), this woody invasive plant has the potential to become problematic over wide areas in Southern Europe as well as in other areas with Mediterranean-type of climate, as suggested by its high expansion in South Africa (van Wilgen & Richardson, 1985; Richardson et al., 1987).

Reports of invasion in Portugal and on the South African fynbos biome – where *H. sericea* causes several negative impacts (Esler et al., 2010) – and of naturalization in France (Brunel et al., 2010) mean that the species is invasive in, or at least has been introduced to, regions within two global biodiversity hotspots, both featuring Mediterranean-type of climate. Therefore, invasion by *H. sericea* may represent a threat to conservation in areas of worldwide importance. Moreover, *Proteaceae* have been recognized as an important group for study in

invasion science (Kueffer et al., 2013), as this group has few invasive species, even though a large number of species have been introduced worldwide (Moodley et al., 2013).

This study was aimed at understanding the current distribution of *Hakea sericea* and anticipating future areas of invasion, thereby contributing to a more effective management of this problematic invader. A sequential, two-scale modelling framework was applied, combining an information-theoretic approach and ensemble modelling to two nested areas and using two different spatial resolutions. The workflow involved three sequential steps. First, the spatial and ecological patterns of *H. sericea* invasion in the two nested areas (western Iberian Peninsula, and northwest Portugal) were modelled. This allowed testing competing hypotheses of which environmental predictors control landscape invasibility at different scales. Finally, the relation between the potential distribution of *H. sericea* and fire occurrence was explored in more detail, using a fine scale fire database available for Portugal (ICNF, 2010).

2. Methods

2.1. Study area and test species

2.1.1. Study area

The study area is composed by two nested geographic areas: a regional study area composed by Portugal and Galicia (Figure 1a, b) with approximately 133 000 km^2 and a local scale study area in the Minho region, with approximately 3086 km^2 (Figure 1a, c). The area is located in the western part of the Iberian Peninsula (Figure 1a) in the transition between the Eurosiberian and Mediterranean biogeographic regions, with most of Galicia and the northwest of Portugal representing the Eurosiberian portion (Rivas-Martínez et al., 2004). The two represented biogeographic regions, along with factors such as high variability in topography, geology and land cover, reflect in a highly heterogeneous area.

The local scale study area (Minho) is a particularly well-studied and data rich area, for both species occurrence and environmental data, owing to several previous scientific publications (e.g. Vicente et al., 2010, 2011; Fernandes et al., 2014), supporting its choice for the local scale component of this work. As this area presents high climatic heterogeneity, with gradients that constrain not only invasive species richness (Vicente et al., 2010), but also *Hakea sericea* potential distribution (see results section), the methodology presented by Vicente et al., (2010) was applied to obtain a climatically more homogeneus area and allow other important factors to be identified as well (for more details see Model Calibration section).

2.1.2. Test species and occurrence data

Hakea sericea Schrad. (Silky hakea) is a divaricate shrub up to 4m high (Barker et al., 1999). It is native to South-eastern Australia, where it can be found in areas of dry sclerophyll forest and heathlands, on coastal regions and adjacent ranges, from the southeast of Queensland to the southeast of New South Wales (Barker et al., 1999). *H. sericea* has a long history of invasion and control attempts in South Africa (Gordon, 1999; Esler et al., 2010). It was introduced to Portugal in 1971 (Almeida & Freitas, 2006) where it became highly invasive in several areas, having spread to at least one location in Spain (Sañudo, 2006). It is now listed in Portuguese legislation as an invasive species (Ministério do Ambiente, 1999), and in Spain as a species with invasive potential (España, 2011).

Some features of this species (detailed in appendix IIId) relevant for its invasiveness are the presence of proteoid roots, that seem to have an important role in nutrient absorption in nutrient poor soils (Sousa et al., 2007), and serotiny (generally understood as prolonged retention of seeds in the canopy; Lamont et al., 1991), usually viewed as a fire adaptation (Weston, 2007). Also, *H. sericea* seeds are stored in woody follicles, with the fruit's thick woody walls offering protection to the seeds against heat (Brown & Whelan, 1999) and

granivores (Groom & Lamont, 1997). Together with a slow decrease in germination rates over time (Richardson et al., 1987; Lamont et al., 1991; Brown & Whelan, 1999), this results in a large canopy-stored seed bank formed by seeds produced in a given year and viable seeds from previous years, which are released after the death of the plant (Richardson et al., 1987). Therefore, an event such as a wildfire results in the sudden release of a very large number of seeds.

Occurrence records were obtained from previous published works, and from the *invasoras.pt* citizen science project (invasoras.pt, 2014), 53 presence records for the regional scale (10x10km cells) and 109 records for the local scale (37 presences and 72 absences – 1x1km cells)

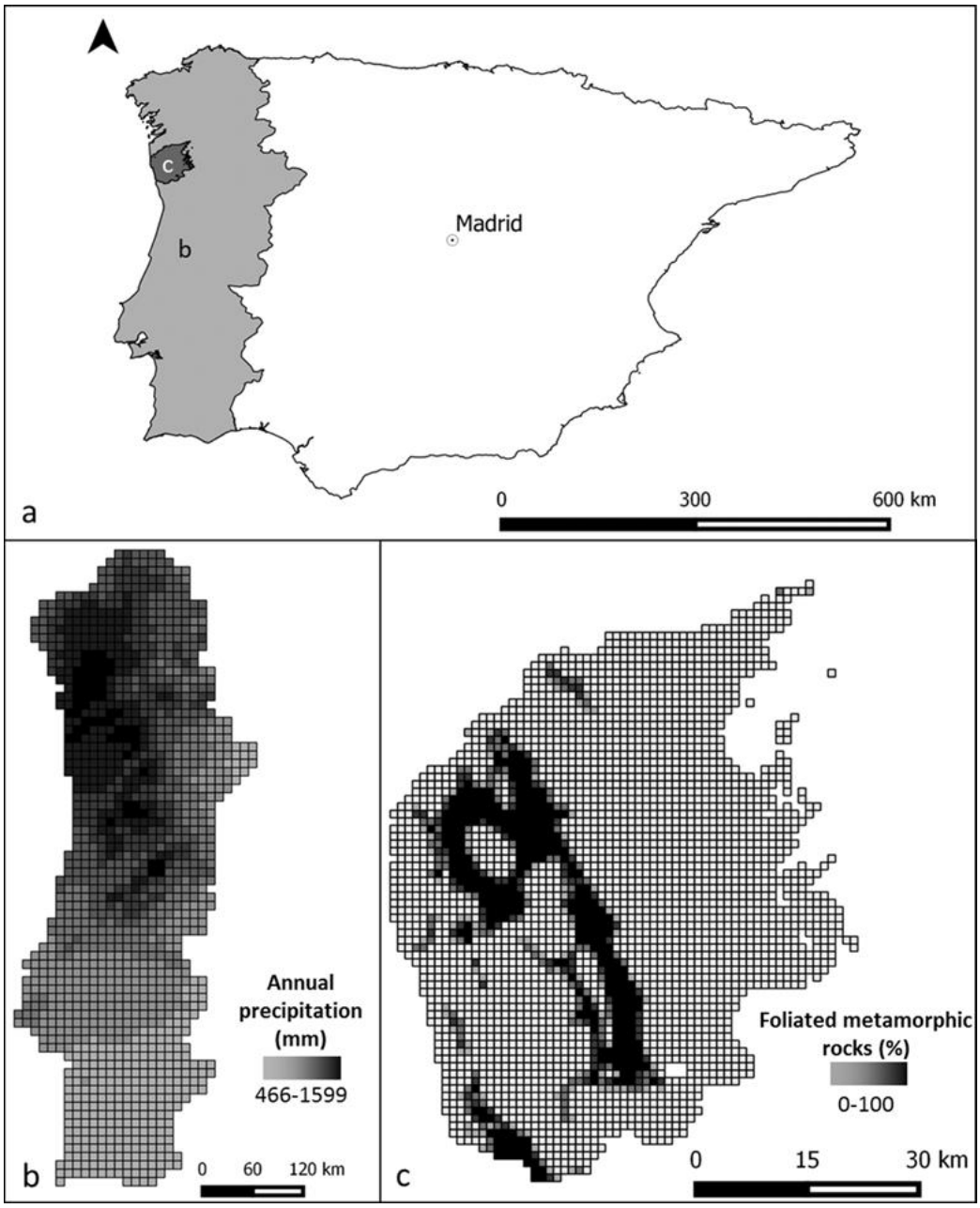

Figure 1 – Study area in the context of the Iberian Peninsula (a). Regional scale study area with values of annual precipitation (b) and local scale study area with values of the percentage area of foliated metamorphic rocks per cell (c).

2.2. Analytical framework

Analyses were performed following the scheme represented in Figure 2. From an initial dataset of environmental variables (a), a set of 16 were selected (b), divided among four sets (climate, landscape composition, landscape structure and geology). These, together with a dataset of presence records and multiple sets of randomly selected pseudo-absences, were used in a multimodel analysis (c) to determine which sets were most important in explaining the distribution of *Hakea sericea* in the regional scale study area, and to perform a spatial projection of the species' distribution (d). This analysis was also used to select the local scale study area, by downscaling (c). For the local scale study area, a similar multimodel analysis was first used to determine the variable importance by set (c, d). As more occurrence records were available for this area, it was feasible to use an ensemble modelling framework with the *biomod2* software (c). This was used to obtain a variable importance score for each variable, and to project the potential distribution of the species for the local scale study area (d). This spatial projection was then used, together with a dataset of fire occurrence, to analyse the relation between the species predicted distribution and the occurrence of fire in the local scale study area (e).

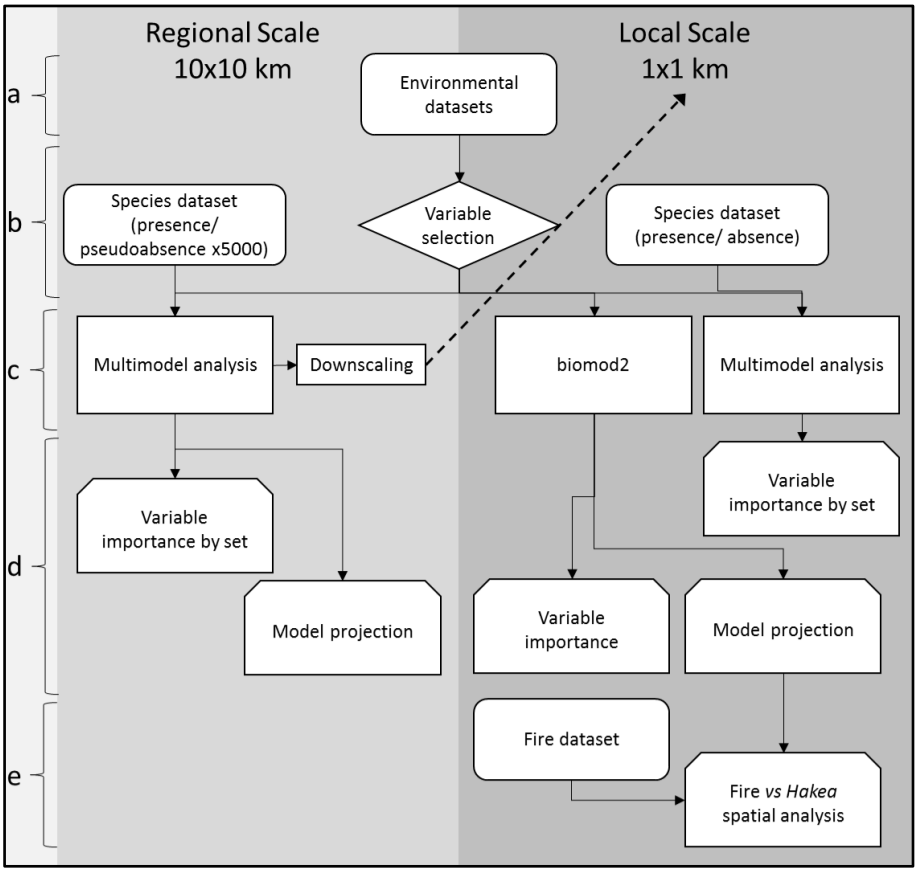

Figure 2 – Analytical framework. Analysis was performed at two spatial scales, with multimodel analysis being used at the regional scale to infer the importance of each set of variables in determining the distribution of the species, for spatial projection, and in a downscaling exercise to establish a local scale study area. In this area, an ensemble model was calibrated using *biomod2*, and variable importance was estimated. Finally, the spatial projection of the ensemble model (as presence/absence) was used to analyse the relationship between the distribution of *Hakea sericea* and the occurrence of fire in the local scale study area.

All the statistical procedures throughout the research workflow were performed using R 3.1.0 (R Development Core Team, 2014). Spatial operations were performed using ArcMap 10.2 (ESRI, 2014) and QGIS 2.2 (QGIS Development Team, 2014).

2.3. Model calibration and evaluation

Table 1 - Variables used for model calibration by variable group, their description and ecological rationale for their choice.

Class	Variable	Description	Ecological rationale
Climate	MinTemp	Minimum temperature of the coldest month	Climate is expected to be the main factor in shaping species' distribution at large scales (Pearson et al., 2002), and previous studies have indicated climate predictors as the most important determinants of invasive species richness in our local scale study area (Vicente et al., 2010).
	TempRan	Temperature annual range	
	AnnPrec	Annual precipitation	
	PrecSea	Precipitation seasonality (Coefficient of variation)	
Landscape composition	pUrbanA	Urban areas cover (percentage)	Land cover/use determine suitable habitat availability, thereby controlling alien invasion, and more invasive species find suitable conditions in man-made habitat (Song et al., 2005). Also, the greater the compositional diversity of a landscape, the more alien invasive species can find suitable conditions there (Pino et al., 2005).
	pAgrico	Agriculture cover (percentage)	
	pArtFor	Artificial forests cover (percentage)	
	pShrubs	Shrubland cover (percentage)	
Landscape structure	NumPatc	Number of patches	By determining ecotone density and diversity, patch shape and size control landscape invasibility (Le Maitre et al., 2004; Dufour et al., 2006).
	mShaInd	Mean Shape Index	
	mPerAreR	Mean Perimeter-to-Area Ratio	
	shDiInd	Shannon Diversity Index of Land Cover classes	
Geology	IgnRock	Felsic and intermediate Igneous Rocks (percentage)	The test species has adaptations related to nutrient absorption. We expect that this might be reflected in differential suitability and invasive potential among different bedrocks, an expectation supported by empirical observations in some locations within our study area.
	MetRock	Foliated metamorphic rocks (percentage)	
	DetSedD	Detritic sedimentary rocks and superficial sedimentary deposits (percentage)	
	geolSDI	Shannon Diversity Index of geological categories	

2.3.1. Variable selection

The selection started with a 65 variable regional scale dataset reflecting four main categories of environmental variables: climate, landscape composition, landscape structure

and geology. Based on previous ecological knowledge of the species and the study area, and applying a correlation analysis, a final dataset of four (Spearman correlation ≤ 0.7) environmental variables per category was obtained. To allow comparability across scales, a dataset with equivalent variables was set for the local scale. The final 16-variable dataset is summarized in Table 1.

2.3.2. Model calibration, evaluation and spatial projection

2.3.2.1. Regional scale modelling

One of the known risks of using SDMs to obtain potential species distributions is overfitting, especially when the number of explanatory variables is too high when compared to the species occurrence data (Guisan & Thuiller, 2005). Instead of fitting a single model using all variables, an information-theoretic approach can be applied, by calibrating a set of competing models reflecting different hypotheses, and then using an adequate metric to compare the power of the several models. Based on an information-theoretic background (Burnham & Anderson, 2002), Multimodel Inference (MMI, appendix IIa) allows the comparison of different scientific hypotheses, by comparing and ranking competing models. Moreover, by weighting each model by its importance to explain the original dataset (i.e. model averaging), it is possible to create a spatial projection, as performed in Vicente et al. (2010). This procedure allows obtaining robust spatial projections while using a large set of environmental variables (Vicente et al., 2010).

We therefore applied a MMI Framework to understand the relative importance of each set of predictors on the distribution of *Hakea sericea* in the regional scale study area, by fitting Generalized Linear Models (GLMs) with each predictor set and using the Akaike Information Criterion (AIC) to assess how well each model is supported by the initial occurrence data. An adaptation of AIC for small sample sizes (AIC_C; Shono, 2000) was used. From the AIC_c we calculated, for each candidate model, the AIC_c difference ($\Delta i = AIC_{c\ initial} - AIC_{c\ minimum}$), a value that allows for comparisons among models. Finally, the Δi values were used to derive Akaike weights (w_i), representing the likelihood that a given model is the best approximating model, given the model and data sets. To assess the models' explanatory power, we calculated Nagelkerke's R^2 (Nagelkerke, 1991). The w_i values were used as weights to obtain an average model, by averaging all competing models. This average model was then used for spatial projection.

Since presence-only information was available for the regional study area, and GLM calibration requires absence information, pseudo-absences, randomly selected from the study area, were used. To avoid the risk of the model being skewed due to the pseudo-absence selection, a bootstrapping procedure, where the full presence set was used together with a random set of pseudo-absences (with the same size as the presence set) was applied to

calibrate the GLMs, with this process repeated 5000 times (each time with a different pseudo-absence set with 53 pseudo-absences, the same number as the number of presence records). The AIC_C and related values were calculated for each repetition, and averaged over the 5000 repetitions.

Besides the four models representing different hypotheses, a null model (intercept model) was also calibrated assuming that all cells had the same probability of occurrence for the species. As such, the model implies that no effect exists from the considered hypotheses over the species' distribution, and its inclusion in the model set allows to test whether the remaining competing models did provide better results than a model considering the absence of climatic, landscape and geological effects.

Model evaluation was performed with the widely used Area Under the Receiver Operating Characteristic curve (ROC-AUC). The model predictions were finally converted into presence/absence using a ROC plot-based approach, by choosing the threshold value corresponding to the point where the ROC curve is closest to the (0, 1) coordinates, as discussed in Liu et al. (2005). ROC plotting and examination was performed with the pROC R package (Robin et al., 2011).

Finally, the average model was used in the selection of the local scale study area, by applying a direct downscaling framework (e.g. Araújo et al., 2005; Fernandes et al., 2014), by projecting the regional scale model to the Minho region.

2.3.1.2. Local scale modelling

A Multimodel Inference Framework similar to the regional scale study area was applied to compare the relative importance of each set of environmental predictors in explaining the potential distribution of the test species. In the local scale area both presence and absence records were available, so no pseudo-absences were selected.

The availability of more occurrence information for *Hakea sericea* in the local scale study area allowed to use an ensemble modelling framework using the whole 16-variable dataset. Ensemble models (appendix IIb) are obtained by calibrating a range of different models (e.g. using different modelling techniques) and combining them into a single forecast. Measurements of model accuracy may aid in this combination, by weighting each models' prediction so that its contribution to the final forecast is proportional to the models' accuracy (Araújo & New, 2007).

Table 2 - Variables used in each of the models calibrated for multimodel inference. Variables are represented in the columns, and models in the lines. The first four models reflect each set of environmental variables, and the last (Null Model) is a random model used for comparison purposes.

	MinTemp	TempRan	AnnPrec	PrecSea	pUrbanA	pAgrico	pArtFor	pShrubs	NumPatc	mShaInd	mPeAreR	shDiInd	IgnRock	MetRock	DetSEdD	geolSDI
M1. Climate	■	■	■	■												
M2. Landscape composition					■	■	■	■								
M3. Landscape structure									■	■	■	■				
M4. Geology													■	■	■	■
M5. Null Model																

Ensemble models are a way to overcome the fact that different modelling techniques can yield very different results even when models are calibrated with the same occurrence and environmental data (Pearson et al., 2006), and that a huge panoply of modelling techniques are available (Araújo & New, 2007; e.g. Guisan et al., 2002; Olden et al., 2008). Ensemble modelling frameworks may then be used to overcome the variability in predictions by allowing the use of multiple modelling techniques (Araújo & New, 2007). This approach has been successfully implemented in *biomod2* (Thuiller et al., 2009), a software package for the R environment allowing the use of 10 modelling techniques to obtain ensemble models and forecasting under current and future environmental conditions.

A total of 310 models were calibrated, using the 10 available modelling techniques. Model evaluations were performed using the AUC value. *Biomod2* uses a cross-validation procedure for model evaluation, where a portion of the dataset is used to calibrate the models while the rest is withheld for evaluation (set to 80% / 20% in our work), with this process being repeated a selectable number of times (30 in our work) plus once with the full dataset.

The ensemble model was obtained by stacking model predictions of all models with an AUC above 0.7, using a weighted approach. In this approach, the weights are proportional to each models' AUC, making each models' contribution to the ensemble proportional to its evaluation metric.

The importance of each variable was estimated for the ensemble model prediction using *biomod2*, by shuffling the values of the variable, projecting the model with the shuffled variable, and finally comparing this projection with the original projection. The variable importance is measured as one minus the correlation between the two projections.

2.4. *Hakea sericea* distribution and fire occurrence

Traits of *Hakea sericea*, such as serotiny, usually viewed as a fire adaptation (Weston, 2007), and the effects over fire related variables the species has been described to have (van Wilgen & Richardson, 1985), led us to hypothesize the existence of a spatial relation between the distribution of the species and fire occurrence. To test this, the relation between the distribution of *H. sericea*, as predicted by our model, and the incidence of fire was examined. This analysis was performed on a dataset of maximum burnt area per cell (as a percentage of cell area), between 1990 and 2009. The model's spatial projection was used to separate this dataset into two groups, one composed of the cells where our species is predicted as present and the other of cells with predicted absence. Differences between the groups were analysed both by visual examination of the variable's distribution in each group, and by performing a statistical test (Wilcoxon rank sum test) comparing the two groups' distributions.

3. Results

3.1. Regional determinants and potential distribution of *Hakea sericea*

Values of w_i for the regional scale models are presented in Table 3. The highest value of *wi* was obtained in the climatic model (M1; w_i = 0.820). The second best model, as supported by the occurrence information, was the one related with landscape composition (M2; w_i = 0.174). The null model was the one presenting the lowest values of w_i (M5, wi=1.91E-06).

Table 3 - Results of Multimodel Inference for the Regional and Local study areas, and variable importance calculated in *biomod2* for the local scale study area.

	Regional MMI		**Local MMI**		***Biomod2* ensemble**	
	w_i	R^2	w_i	R^2	**Variable**	**Importance**
M1 – Climate	**0.820**	0.548	**0.278**	0.346	TempRan	**0.331**
					PrecSea	**0.107**
					MinTemp	0.039
					AnnPrec	0.023
M2 – Landscape composition	**0.174**	0.444	**0.722**	0.361	pShrubs	**0.214**
					pUrbanA	0.057
					pArtFor	0.051
					pAgrico	0.024
M3 – Landscape structure	0.005	0.264	1.04E-07	0.117	shDiInd	0.084
					NumPatc	0.024
					mShaInd	0.011
					mPeAreR	0.003
M4 – Geology	0.001	0.181	5.41E-06	0.178	MetRock	**0.628**
					IgnRock	0.026
					DetSEdD	0.021
					geolSDI	0.015
M5 – Null model	**1.91E-06**	0.008	**8.25E-09**	0.021		

The current potential distribution of *Hakea sericea* in the regional study area is shown in Figure 3a. Of a total of 1330 grid cells, 304 (22.9%) are predicted as potential presence, and the remaining 1026 (77.1%) as absence. The potential presences are located mostly in the western part of the area, near the coast, with most of them being limited to the western half of central and northern Portugal, extending northward to southwest Galicia. Evaluation of the projection of the averaged model, for the full regional scale study area, indicates very good predictive power (AUC = 0.8825).

3.2. Local determinants and potential distribution

Results of multimodel analysis for the local scale study area are presented in Table 3. The model with highest w_i is M2 (Landscape composition, w_i = 0.722), followed by M1 (Climate,

w_i = 0.278). The remaining models have low scores, with the null model having the lowest (M5, w_i = 8.25E-09)

Variable importance values from *biomod2* are presented in Table 3. The variable with the highest value was the percentage of foliated metamorphic rocks (MetRock = 0.628). This geological variable was followed in importance by variables reflecting climate and landscape composition, namely temperature annual range, percentage of shrubland cover and precipitation seasonality (TempRan = 0.331, pShrubs = 0.214, PrecSea = 0.107).

The model projection for the local scale study area is presented in Figure 3b. A total of 109 cells (3.5%) are predicted as presences and 2977 (96.5%) as absences, of a total of 3086 cells. The majority of the predicted presences are concentrated in the north-western quarter of the study area, in a zone marked by the presence of schist (Figure 1c). Evaluation of model performance indicates very good predictive power (AUC = 0.9).

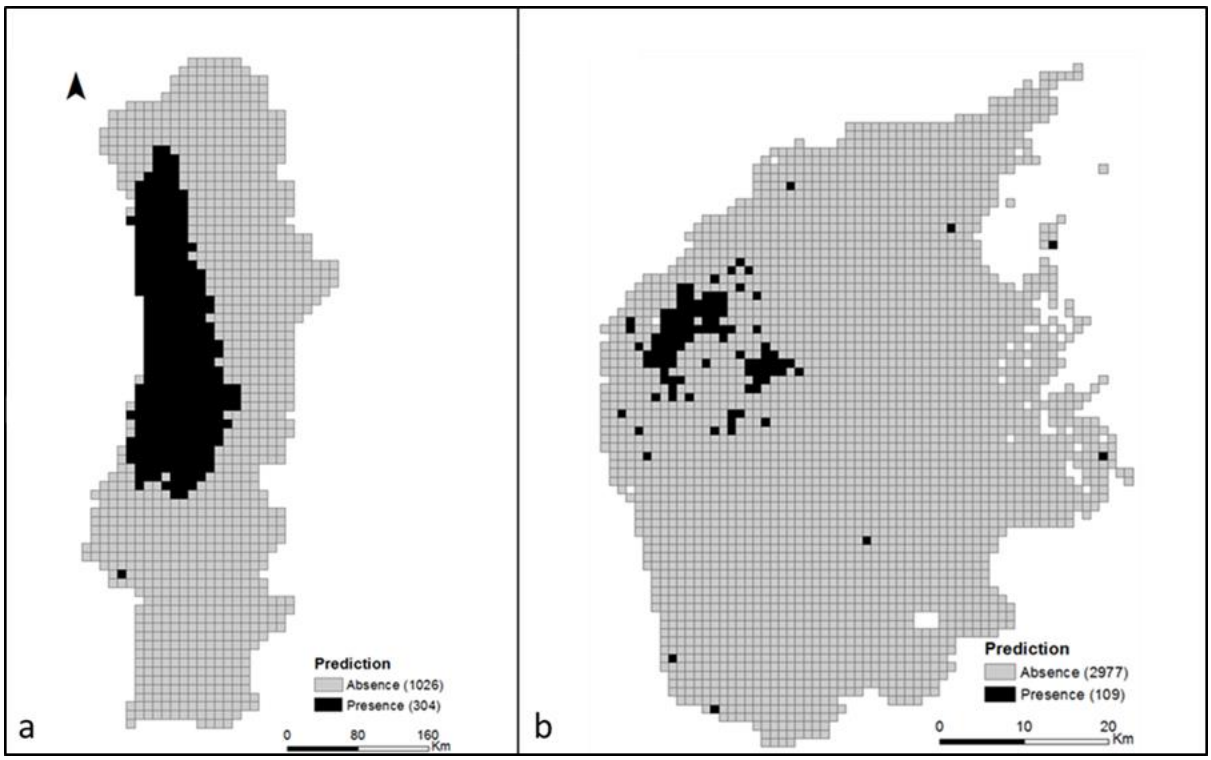

Figure 3 - Potential distribution of *Hakea sericea* in the regional scale study area (a), obtained by binarization of the average model projection, and in the local scale study area (b), obtained as an ensemble forecast in *biomod2*.

3.3. *Hakea sericea* distribution and fire regime

Figure 4 presents the comparison of the maximum burnt area per 1km^2 cell, for the cells predicted as presence or absence for *Hakea sericea*, showing a significant difference between the two groups ($p<0.05$). A clearly larger burnt area is significantly related with the predicted presence cells (median = 68.01%), compared to the cells predicted as absence median = 7.81%).

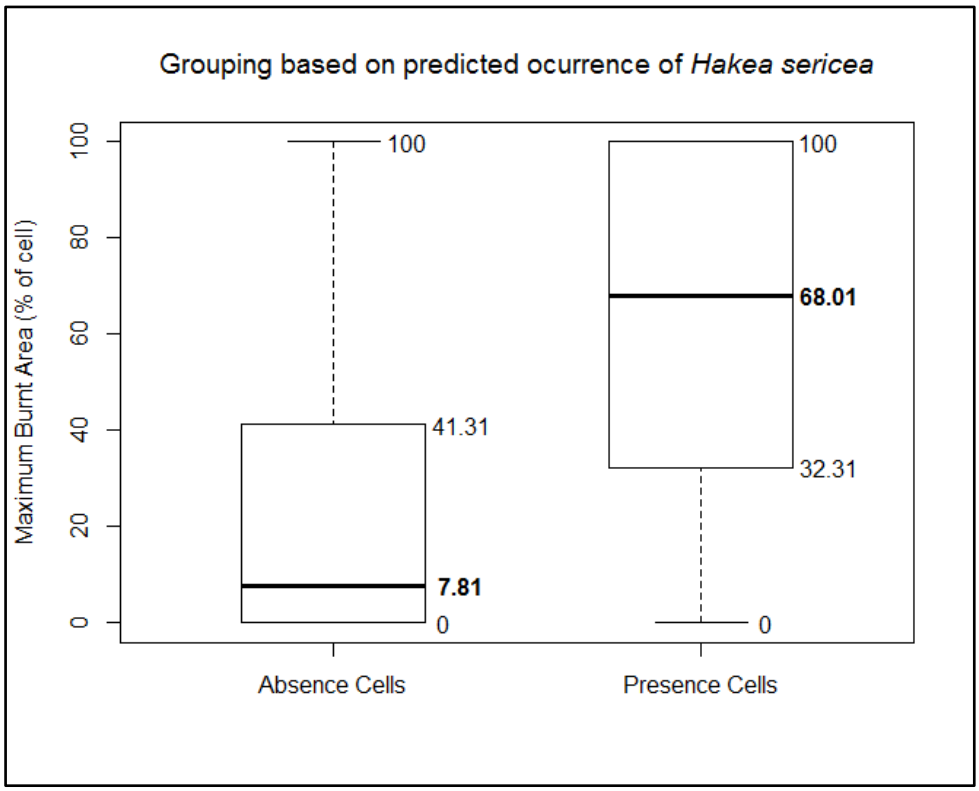

Figure 4 - Boxplots of the maximum burnt area per cell (percentage) for cells where *Hakea sericea* is predicted to be absent (left) or present (right). Values represent minimum, first quartile, median, third quartile and maximum. On the absence cells' boxplot the minimum and the first quartile are both zero, while in the presence cells' boxplot the third quartile and maximum are both 100.

4. Discussion

4.1. Determinants of *Hakea sericea* distribution and future expansion

The importance of different types of environmental predictors to explain species distributions varies across spatial scales, with climate being of primary importance over large scales and factors such as land cover increasing in importance towards local spatial scales (Pearson et al., 2004; Guisan & Thuiller, 2005). By applying a multiscale approach to the study of invasion by *Hakea sericea*, the results presented here show that, in Western Iberia, the importance of different sets of drivers in determining the species' distribution varies over spatial scales. Considering a regional scale comprising Portugal and Galicia, the climatic model was the most relevant for explaining the data. Climate is known to be the most important factor in shaping the distribution of species over large scales (Pearson et al., 2002), and our results support this idea. Species of subtropical and lowland origin, like *H. sericea*, are limited in the study area by frost sensitivity, being excluded from mountain landscapes (Vicente et al., 2010). With future climate changes, this climate constraint is expected to become more limited (Walther, 2002), and consequently these areas may become susceptible to invasion.

Landscape traits have been reported to be important in determining invasion (Pino et al., 2005; Vicente et al., 2010), and are known to be related to climate (Thuiller et al., 2004). Accordingly, landscape composition was also important in explaining the current distribution of *H. sericea* at the regional scale. When the local scale was tested, the competing models ranking was the opposite of the regional scale, with the landscape composition model being the most important, followed by the climatic model. This, is, again, in agreement with predictor importance variation across scales, and with the increasing importance of land cover/use with decreasing spatial scale (Pearson et al., 2004; Guisan & Thuiller, 2005; Vicente et al., 2010). Future shifts in land use could thus drive expansion (or contraction) of *H. sericea* range at both scales considered.

Ranking the environmental variables importance in *biomod2*, a single environmental geology-related variable was selected as the most important to determine *H. sericea* distribution at the local scale. This geological variable (percentage of foliated metamorphic rocks) reflects a geological class (schist) that, together with granite (reflected in our set by the percentage of felsic and intermediate igneous rocks variable), dominates the geological composition of the local scale study area. Previous empirical observations had already indicated that, for this area, the species shows a clear preference for areas with a schist bedrock and, considering our results and the fact that *H. sericea* shows adaptations for nutrient absorption (Sousa et al., 2007), we hypothesize that this adaptations might be an important factor in causing the differential preference observed both empirically and in models. The existence of native species that, in the study area, have similar preference for areas with schist

bedrock, such as the western Iberian endemism *Succisa pinnatifida* Lange, makes this result particularly important, as conflicts between invasive and rare species have already been forecast to occur in the study area (Vicente et al., 2011).

4.2. *Hakea sericea* and fire regime

Invasive species are known to be able to benefit from, and to impact, disturbance regimes (Mack & D'Antonio, 1998), and, with particular relevance in this situation, fire regimes (Brooks et al., 2004). In this study, the spatial projection of *Hakea sericea* was overlaid with fire occurrences in the local scale study area. Our results show a significant relation between the areas predicted as presence for the invasive species and the highest incidence of burnt areas.

Alien invasive species can modify feedback mechanisms of ecosystems, an issue that has recently received much attention in the scientific literature (Brooks et al., 2004; Suding et al., 2013; Gaertner et al., 2014). *H. sericea* has been observed to cause changes in fire related variables in some environments (van Wilgen & Richardson, 1985), suggesting that the presence of *H. sericea* may be promoting fire proneness in the invaded study area. Moreover, *H. sericea* is a fire adapted species, with seeds being stored and protected (Groom & Lamont, 1997; Brown & Whelan, 1999) throughout the life of the plant and released in large quantities at the plant's death (Richardson et al., 1987). Therefore, an event such as a wildfire results in massive propagule release, with a large number of seeds being released from a large number of individuals. We expect that both these mechanisms may play a role in the observed differences in burnt areas, with *H. sericea* both favouring the occurrence of fire, and being favoured by it, suggesting a change in ecological feedback mechanisms towards increased fire proneness.

Considering the scientific literature together with our results, we believe that feedback modification is taking place in our study area, constituting a starting point for future research, to shed more light on the nature of the relationship between *H. sericea* and fire in particular, and to contribute to the understanding of the effects of invasion over disturbance regimes and ecosystem feedback mechanisms in general. Management options have been proposed for species that cause, or have the potential to cause, shifts in fire regimes (Brooks et al., 2004), and our results suggest that these should be taken into account in management of *H. sericea* invasion.

4.3. Implications for management of *Hakea sericea* invasion in Mediterranean landscapes

Based on predictions from our multiscale modelling of the spatial patterns of *Hakea sericea* invasion and its ecological drivers, at two spatial scales, we found variations on the importance of environmental drivers across scales. At the regional scale, climate was the most important driver of the species distribution, followed by landscape composition. At the local scale this was reversed, so drivers are scale-dependent. Moreover, the most important single variable at local scale was a geological variable, which is likely related with specific adaptations to nutrient absorption in *Hakea*. Rare species of high conservation importance present in our study area show a similar preference, raising concerns about possible conflicts with implications for conservation strategies.

Since fire and invasion by exotic species are two of the most important disturbances in areas with Mediterranean-type of climate (Pausas et al., 2008; Gaertner et al., 2009; Brunel et al., 2010), our results showing that invasion by *H. sericea* may lead to changes in fire regimes are particularly important for management of both types of disturbances in Mediterranean landscapes. Preventing or managing landscape invasion by *Hakea* could play an important role in fire prevention and risk management, especially in heavily invaded regions. This would in turn contribute to a more effective control of *Hakea* in invaded regions.

Prevention efforts are known to be the most cost-effective measures to control invasions (Davies & Sheley, 2007), and therefore the spatial projections of the potential distribution of *H. sericea* presented in this study should be taken into account when developing early detection and invasive species management plans, particularly for Galicia (northwest Spain), being a not yet invaded but potentially suitable area. Also, we argue that invasive species management must be considered across borders (for example as a coordinated European strategy, e.g. European Comission, 2008; Hulme et al., 2009) as prevention and/or control plans can only be effective if the whole potential distribution of the species is considered.

H. sericea is already considered as a problematic invasive species in Portugal and as a species with invasive potential in Spain (Ministério do Ambiente, 1999; España, 2011). This is supported by our results, which indicate as suitable area for the species an area of Galicia (northwest Spain) considerably larger than the current known occurrences. Our spatial projections and the scale-dependence of key determinants of *Hakea* invasion support the idea that trans-border action must be considered when effectively dealing with this and other invasive species.

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Appendix I. Invasive species – concepts and definitions

Alien species (also called adventive, exotic, foreign, introduced, non-indigenous, or non-native) are species that, through human agency, are able to overcome biogeographic limitations to dispersal, and become present in a region outside their native range (Richardson et al., 2011). Invasive species may be defined as "Alien species that sustain self-replacing populations over several life cycles, produce reproductive offspring, often in very large numbers at considerable distances from the parent and/or site of introduction, and have the potential to spread over long distances […]" (Richardson et al., 2011, p.415). The process by which a given species achieves such a status may be conceptualized in a series of stages, separated by barriers that the species/population must overcome (Richardson et al., 2000; Blackburn et al., 2011) (Figure I.1).

An invasive species is, by definition, an **alien species**. That is, it was able to overcome a geographical barrier due to human action (intentional or non-intentional), becoming present in a region outside its natural range. In the case of intentional introduction, a second barrier may be considered (Blackburn et al., 2011), the "barrier of captivity and cultivation", in this case a conceptual barrier that may have a physical expression – for example, a species may be introduced into cultivation but stay physically enclosed by humans, and therefore unable to spread. Then, invasive species must "sustain self-replacing populations over several life cycles, produce reproductive offspring, often in very large numbers". This implies overcoming two new barriers: survival and reproduction. First, the species needs to be able to survive the abiotic and biotic conditions in the area of introduction, becoming a **casual species**. However, conditions "good enough" to allow for survival may not be sufficient for reproduction, therefore, a species will only be able to establish a self-sustaining population and become a **naturalized species** after overcoming both the barriers of survival and reproduction. Finally, invasive species' offspring is produced "at considerable distances from the parent and/or site of introduction, and [the species] have the potential to spread over long distances". It is this ability to overcome barriers to dispersal and survival in the colonized environments, establishing populations far away from the original introduction site(s) that defines **invasive species**.

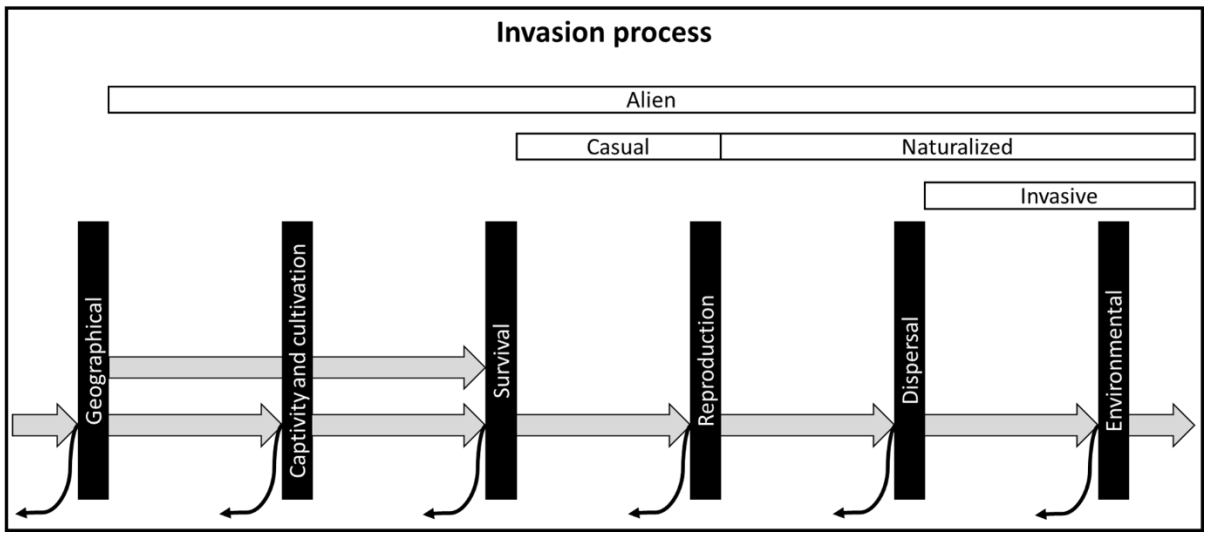

Figure I.1 - Conceptualization of the invasion process. Black rectangles represent barriers to invasion, and grey arrows indicate the progression of species along the process. The small black arrows imply that failure to surpass any of the barriers means a failure to invade. The white rectangles indicate the designations of species in different phases of the invasion process. The grey arrow bypassing the cultivation barrier indicates that many species, being accidentally introduced, are introduced straight into the new environment, not being subjected to this barrier. Based on Richardson et al (2000) and Blackburn et al (2011).

While some definitions of invasive species, such as the one presented in Portuguese legislation (Ministério do Ambiente, 1999) and in European proposed legislation (European Comisson, 2013) explicitly consider the importance of invasion impact[1], the definition proposed by Richardson et al. (2000, 2011) does not, focusing instead on the spreading ability of the alien species. For species clearly causing impacts over the ecosystem – for instance on nutrient cycling, water usage or fire regime, there is a specific terminology of classification: **transformer species** (Richardson et al., 2000). It's important to state that while invasive species are alien species, not all alien species become invaders, since most of the alien species introduced over the world never became invasive (Richardson et al., 2011).

Ia. References

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Ministério do Ambiente (1999) Decreto-Lei nº565/99, de 21 de Dezembro. *Diário da República - I Série n.º 295*, 9100–9115.

[1] For example, the proposed European legislation defines alien invasive species as "an alien species whose introduction or spread has been found, through risk assessment, to threaten biodiversity and ecosystem services, and that may also have a negative impact on human health or the economy"

Richardson D., Pyšek P., & Carlton J.T. (2011) A Compendium of Essential Concepts and Terminology In Invasion Ecology. *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (ed. by D.M. Richardson), pp. 409–420. Blackwell Publishing, UK.

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Appendix II. Species Distribution Modelling

Perception, over the course of history, of the existence of an association between the physical environment conditions and the distribution of species led to an interest to describe and understand those associations, with a particular emphasis (arising more recently) on numerical description (Guisan & Thuiller, 2005; Elith & Leathwick, 2009). Species Distribution Models can be defined as methodologies that relate data on species distributions (e.g. presence or absence in a given place) with environmental information (e.g. climatic conditions), providing the ability to achieve the goal of understanding and predicting species' distributions (Elith & Leathwick, 2009). Therefore SDMs have become a very important tool in ecologic studies (Guisan & Zimmermann, 2000).

Over the last decades, SDMs have been increasingly used in the field of invasion biology to (Figure II.1): analyse and explain current patterns of invasion (descriptive models *sensu* Shmueli, 2010; Vicente et al., 2010; Petitpierre et al., 2012); identify key environmental variables driving alien invasive species distribution (explanatory models *sensu* Guisan & Thuiller, 2005; Shmueli, 2010; Vicente et al., 2010); and to predict potential geographic distribution of alien invasive species under current conditions and environmental change scenarios (predictive models *sensu* Araújo et al., 2005; Shmueli, 2010; Vicente et al., 2011).

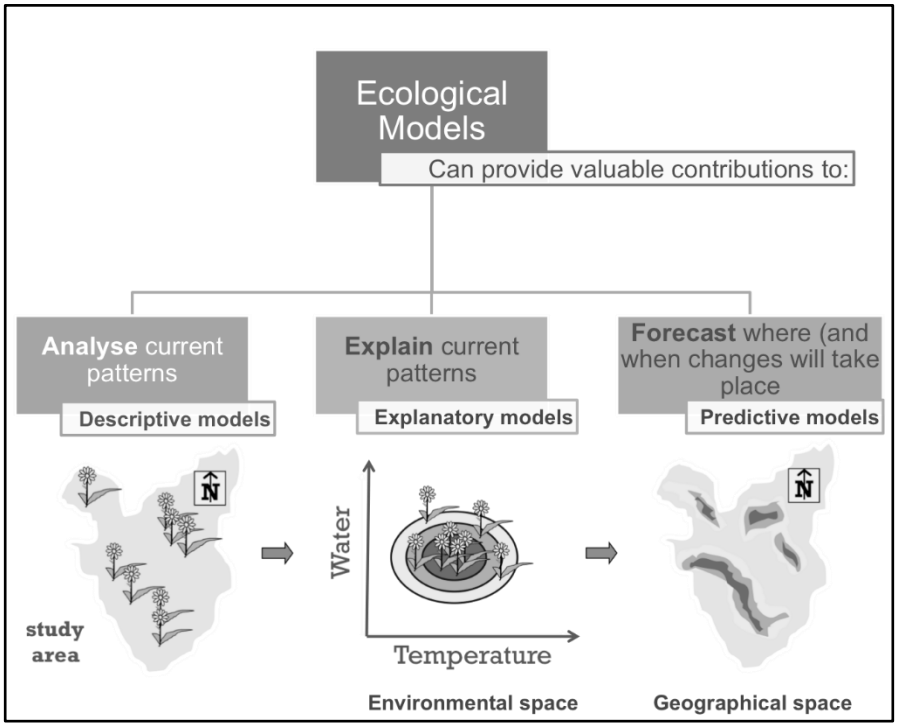

Figure II.1 – Possible applications of Species Distribution Models to invasion research. Graphs courtesy of Antoine Guisan.

Ecological models are based on the simplification of reality, and do not aim at reflecting reality in its entirety, instead focusing on the features relevant for a given question/objective (Anderson, 2008; Jørgensen & Fath, 2011). Moreover, models are fitted with datasets, which

are samples of reality with limited size (in the case of SDMs, the most relevant limitation being the number of available occurrence records). Therefore, limitations arise when calibrating the model, for instance on the selection of the number of parameters (for SDMs, typically environmental variables) that may be included without violating the models statistical assumptions – a very important concept is parsimony. In this context, parsimony is the best choices considered in the modelling procedures to reduce the risks of under- and overfitting. On one hand, selecting a low number of parameters, considering the sample size of the occurrence dataset, can lead to a model that likely misses important information present on the dataset, leading to underfitting. On the other hand, selecting too many parameters considering the sample size of the occurrence dataset incurs on the opposite risk: the model will capture random noise, considering it a feature of reality – overfitting (Anderson, 2008). With models being approximations of reality, it is more useful to think of different models as being "better" or "worse", instead of "right" or "wrong" – a given model may be able to better extract the information contained in a dataset, but that does not mean it is the "right" model (Anderson, 2008). A way to obtain a better model is, given a set of competing models, to obtain the empirical (i.e. given by the data) support of each model, by comparison with the empirical support of the other competing models. Particularly, if each model reflects a different scientific hypotheses, we can assess how the available data supports different scientific hypotheses in comparison to others (Anderson, 2008).

IIa. Multimodel Inference

Information-theoretic approaches such as Multimodel Inference (MMI; Burnham & Anderson, 2002) apply the competing models idea to select and obtain inferences from model sets (Figure II.2). The Akaike Information Criterion (AIC; Akaike, 1973) is an estimator that allows the quantification of the amount of information that is lost when a given model is created to approximate reality. In practice, when a set of models are calibrated, it is possible to identify the model that minimises information loss, by calculating the AIC of the competing models and selecting the model with the lowest AIC value, and therefore the lowest distance to reality. Still, reality is unknown and has to be estimated from the available data for each study – consequently, the selected model will be the best approximation to reality *given the data and model sets*, and, accordingly, the data has to be obtained and the models developed under sound scientific principles (Anderson, 2008).

More than simply selecting the single "best" model in the set, this approach allows the ranking of the several competing models. Under a Multimodel Inference framework inferences are made using all models and environmental variables in the dataset, considering that, even if one model is the best approximation to reality, that does not imply that the other models contain no extra information (Anderson, 2008). From the AIC values, Akaike weights (w_i) can

be derived. W_i indicates the probability of a given model being the best, given the original data (Anderson, 2008). Akaike weights may be used as averaging weights for the competing models, in order to obtain an average model, which can be used for spatial and temporal prediction purposes.

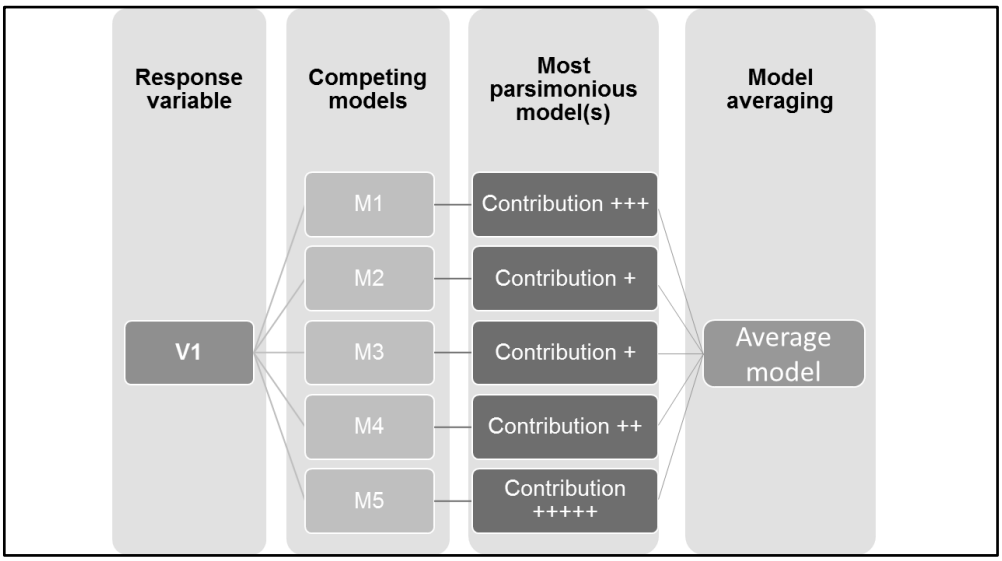

Figure II.2 - Multimodel Inference Framework. A set of models, reflecting different hypotheses, are calibrated. A measure of empirical support for reach model is calculated, based on the information lost on calibration. This measure of support may then be applied to weight an average model, were each models' contribution depends on how well supported by the data it is.

IIb. Ensemble modelling – *biomod2*

Advances in statistical knowledge and computer technology made available a wide range of tools, techniques, and options for statistical modelling, some of them applied to SDM development (Guisan & Zimmermann, 2000; Araújo & New, 2007). SDMs, being correlative models, are very sensitive to the choices related to mathematical functions and to variations in the data (Araújo & New, 2007), leading to situations where different modelling techniques produce very different predictions, even when calibrated with the same original data (Pearson et al., 2006).

Ensemble forecasting (Figure II.3), obtained by fitting a large number of models, using multiple sets of model classes (and of other elements, such as the models' initial conditions or boundary conditions, according to the objectives), and exploring the resulting range of forecasts, are a proposed way of dealing with this inter-model variability (Araújo & New, 2007). *Biomod2* (Thuiller et al., 2009) is a package implemented in the R software that allows the use of ensemble forecasting modelling. *Biomod2* currently incorporates 10 modelling techniques (Table II.).

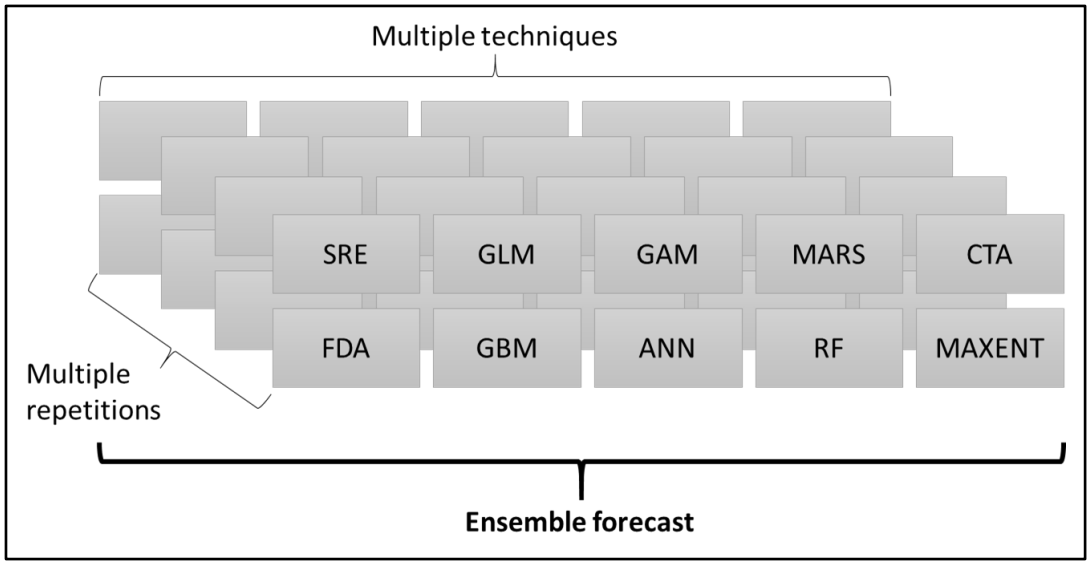

Figure II.3 - Conceptualization of an ensemble model. The ensemble forecast is obtained by combining projections of multiple modelling techniques (10 in *biomod2*), with multiple repetitions of each.

Table II.1 - Modelling techniques available in *biomod2*, grouped by model type.

Model classification	Acronym	Technique
Bioclimatic envelope	SRE	Surface Range Envelope
Regression methods	GLM	Generalized Linear Model
	GAM	Generalized Additive Model
	MARS	Multivariate Adaptive Regression Splines
Classification methods	CTA	Classification Tree Analysis
	FDA	Flexible Discriminant Analysis
Machine learning methods	GBM	Generalized Boosting Model
	ANN	Artificial Neural Network
	RF	Random Forest
	MAXENT	Maximum Entropy

Several model evaluation metrics are available in *biomod2*, such as Cohen's Kappa, True Skill Statistic (TSS), and the Area Under the Receiver Operating Characteristic curve (ROC-AUC), with AUC being one of the most widely used. The ROC curve (Figure II.4) is obtained by selecting a series of threshold values (i.e. cut-off values for the model outputs, defining the threshold between presences and absences) above which the output is considered to be a presence, and under which the result is an absence, and plotting, for each threshold, the true positive rate as a function of the false positive rate (sensitivity versus 1-specificity, or the ratio of correctly predicted presences versus the ratio of incorrectly predicted presences (Fielding & Bell, 1997). Values of sensitivity and 1-specificity vary between 0 and 1, so a "perfect" model that predicted only true presences and true absences would produce a curve encompassing the whole plot, and have an AUC value of 1. The AUC value decreases when the accuracy also decreases, leading to less true positives and more false positives. The incorporation of multiple thresholds in the calculation makes AUC-ROC a threshold-

independent measure of accuracy, as the metric does not depend on the threshold selection. Moreover, the ROC curve can be also used for threshold selection to obtain binary predictions from a probabilistic output, by selecting as threshold the point on the ROC curve closest to the (1, 1) corner (Cantor et al., 1999; Liu et al., 2005), that corresponds to a model with both 100% sensitivity and specificity (Cantor et al., 1999).

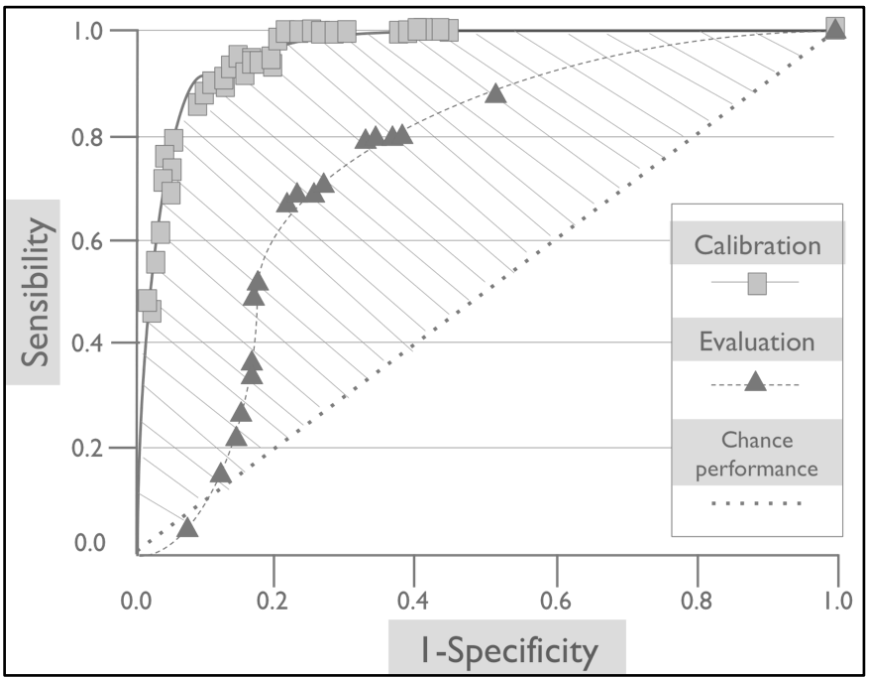

Figure II.4 - Receiver Operating Characteristic plot, with curves plotted for both calibration and evaluation example datasets.

To apply evaluation metrics such as the AUC, a dataset is needed to fit the models, and an independent dataset is needed for evaluation. However, limitations on the size of the available datasets often don't allow the independent data evaluation (Guisan & Zimmermann, 2000). To overcome this problem, several techniques have been developed, such as cross validation. In cross validation the dataset is split into a given number of subsets (Figure II.5 IIa), and one of the subsets is used for evaluation, with the remaining data used for calibration. The extreme of a cross validation technique is jackknifing, where every observation except one is assigned for model calibration (Figure II.5 IIb). Jackknifing is not very often used for evaluation purposes, as it tends to provide overestimated evaluations. The process of calibration/evaluation is repeated several times with all possible different subset combinations assigned for calibration and evaluation.

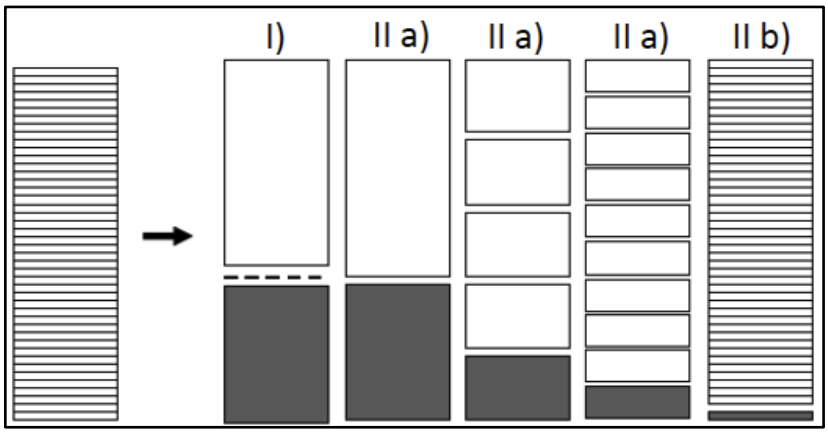

Figure II.5 - Possible choices for model validation. I) using a separate dataset for validation. II) Cross validation, where the dataset is partitioned into calibration and evaluation datasets.

IIc. References

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Appendix III. *Hakea sericea*

Hakea sericea Schrad. (Silky hakea; Figure III.2, Figure III.3) is a divaricate shrub up to 4m high (Barker et al., 1999). *H. sericea* belongs to the *Proteaceae* family, woody plants mostly from the southern hemisphere's Gondwanic continental block, comprising ±1700 species of 80 genera, most diverse in Australia, followed by Southern Africa (Weston, 2007). *H. sericea* is native to Australia and has been introduced and became an invader in several regions around the world. Currently, *H. sericea* has become an aggressive invader in many areas on mainland Portugal, with at least one reported case of spread to Spanish territory (Sañudo, 2006). Some studies using *H. sericea* as test species have already been published in Portugal, mostly related with physiological perspectives (Sousa et al., 2004, 2007; Luís et al., 2014). There's not been, to our knowledge, any attempt to model *H. sericea* potential distribution within the Iberian Peninsula.

IIIa. Distribution – native and introduced

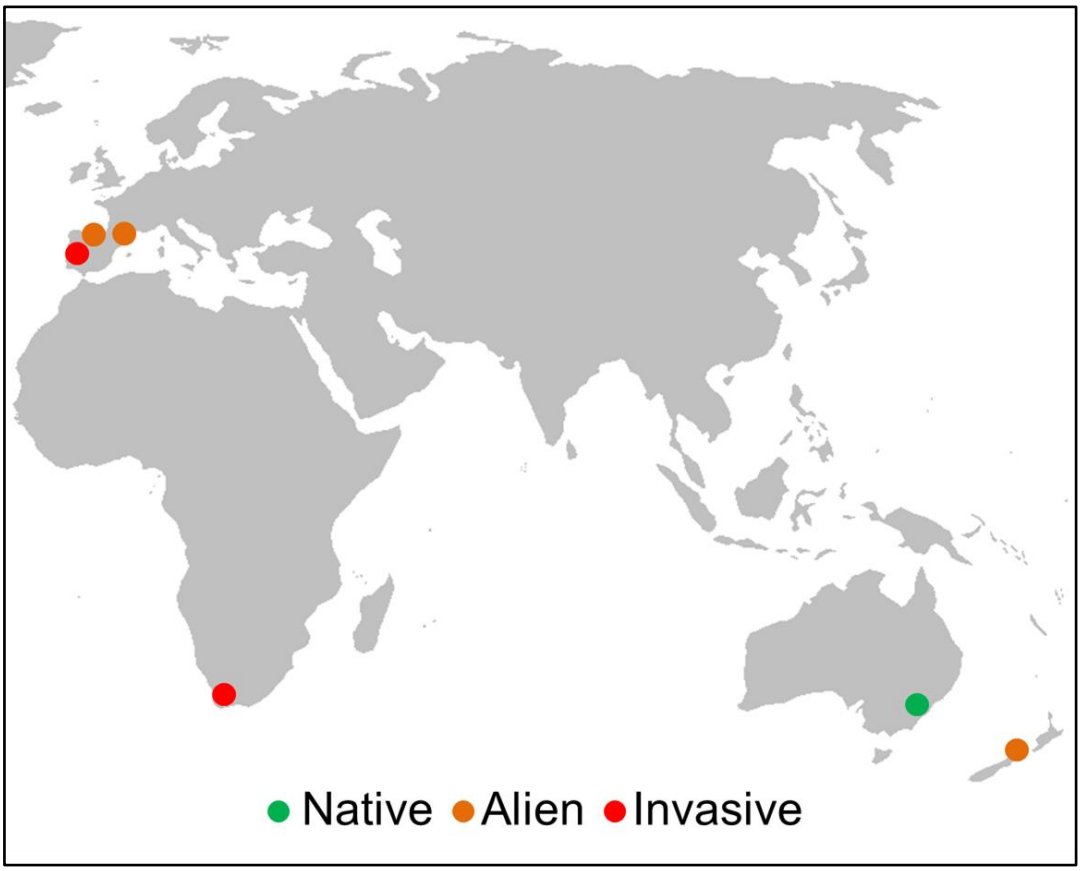

Figure III.5 - Worldwide distribution of *Hakea sericea*. Basemap via Wikimedia Commons (Public Domain).

H. sericea is native to South-eastern Australia (Figure III.1, green dot), where it can be found in areas of dry sclerophyll forest and heathlands, on coastal regions and adjacent ranges, from the southeast of Queensland to the southeast of New South Wales (Barker et al., 1999). In its native region, *H. sericea* is a common species in the vegetation communities

of the Hawkesbury sandstone area (Brown & Whelan, 1999), a geological area commonly found around Sydney and noted as having very low nutrient concentrations, namely of phosphorous and nitrogen (Thomson & Leishman, 2004).

Introductions of *Hakea sericea* have been reported in several regions in both hemispheres, such as in New Zealand, Spain, France (Figure III.1, orange dots), South Africa and Portugal (Figure III.1, red dots). In New Zealand, where it was introduced for hedging purposes, it is now naturalized (Webb et al., 1988), and considered a weed (Howell, 2008). Reports of introduction for hedging and posterior spread also come from nearby Norfolk Island (Green, 1994). Despite the species not yet being listed as occurring in Spain in publications such as Flora Iberica (Castroviejo, 1986-2012), its presence has been reported for at least one area in southern Galicia (Sañudo, 2006). Naturalization in some areas in France is also known (Brunel et al., 2010). There are two herbarium specimens, dated from 1932 and 1964, that place the species in two locations in the Huambo region, Angola (Instituto de Investigação Científica Tropical, n.d.). The South African and Portuguese cases are detailed in the following sections.

IIIb. Invasion and control in South Africa

Four species of the genus *Hakea* Schrad. & J. C. Wendl. were introduced to South Africa between 1840 and 1860 (Macdonald, 1984, cited in Richardson et al., 1987): *Hakea sericea*, *Hakea gibbosa* (Sm.) Cav., *Hakea suaveolens* R. Br. and *Hakea salicifolia* (Vent.) B. L. Burtt., and all but *H. salicifolia* have been declared as noxious weeds since then. *H. sericea*, while not widely planted at the introduction time, became the woody invasive with the largest area of occupation in the fynbos biome (Macdonald & Jarman, 1984, cited in Richardson et al., 1987).

Since the declaration of *H. sericea* as a noxious weed, in 1937, several control approaches have been attempted, starting with mechanical clearing based on simply felling the plants (Esler et al., 2010). However, after the death of the plants, the follicles open and release the seeds, which germinate. Burning the area 12-18 months after felling is a methodology that has “proved very effective”, but it was “not used consistently until the mid 1970s” (Esler et al., 2010, p. 212), having since been used in some campaigns, the largest one in the late 1970s and early 1980s (Esler et al., 2010). In 1970, biological control was initiated, with efforts focused on the use of seed feeding agents, namely the weevil *Erytenna consputa* Pascoe and the moth *Carposina autologa* Meyrick, insects that may be considered to have a complimentary approach, since the weevil attacks developing fruits, while the moth feeds on seeds from mature fruits (Gordon, 1999). A simulation study (Le Maitre et al., 2008) indicated that the presence of these control agents may have a substantial impact over the seed bank, and therefore over the dispersal, invasion rate and population growth rates.

Another biological control approach has relied on the action of a fungus. *Colletotrichum gloeosporioides* (Penz.) Sacc. was identified as a species of fungi causing disease in some stands of *Hakea sericea*, and has posteriorly been proposed for use on biological control actions in South Africa (Morris, 1989), with a commercial mycoherbicide being developed and branded as Hakatak® (Morris et al., 1999). Phylogenetic studies have indicated that the fungus is not *C. gloeosporioides*, but *Colletotrichum acutatum* f. sp. *hakeae* Lubbe, Denman, P.F. Cannon, J.Z. Groenew., Lampr. & Crous, a *forma specialis* that appears to be highly specific to the genus *Hakea* (Lubbe et al., 2004).

The comparison of two surveys (1979, aimed at *H. sericea* and 1991-2001, aimed at *Proteaceae* in general) revealed some interesting trends for *H. sericea*, as reported by Esler et al., (2010). A 64% reduction in the area where the species was recorded (530 000 ha in 1979 to approximately 190 000 ha in 2001), a reduction of 96% in the area categorized as "high density" (from approximately 120 000 ha to 4 500 ha), and, more generally, an area of approximately 490 000 ha were the species either decreased in density or completely disappeared, offer some promising results. Despite that, it is a fact that an area of 107 000 ha had either a new presence recording or an increase in density. Still, the authors recognize a generally decreasing trend in both species density and area of distribution. Two main forces appear to be behind these trends: firstly, the mechanical control with the method of felling and burning appears to have caused a sharp decline in densities. However, this does not explain how this reduction remained, because, after the initial impetus in the 1970s, funding cuts and new management policies have reduced the use of this technique. The relative lack of re-invasion may then be explained by the presence of the mentioned biological control insect agents, as they attack the seeds that would allow for a new wave of spreading and a rise in densities.

IIIc. Portugal

Figure III.2 - Area invaded by *Hakea sericea* in northern Portugal.

In the Portuguese territory, *Hakea sericea* is reported as naturalized both in the mainland (Figure III.2) and in Madeira island, where it was introduced, probably around the 1930's, with ornamental and honey production purposes, as well as to serve as an hedge plant (Vieira, 2002). However, it is in the mainland (Figure III.3) that the species presents a more aggressive invasive behaviour. *H. sericea* is listed as an invasive species in Portuguese legislation (Ministério do Ambiente, 1999), limiting its authorized uses and forbidding introduction in the wild.

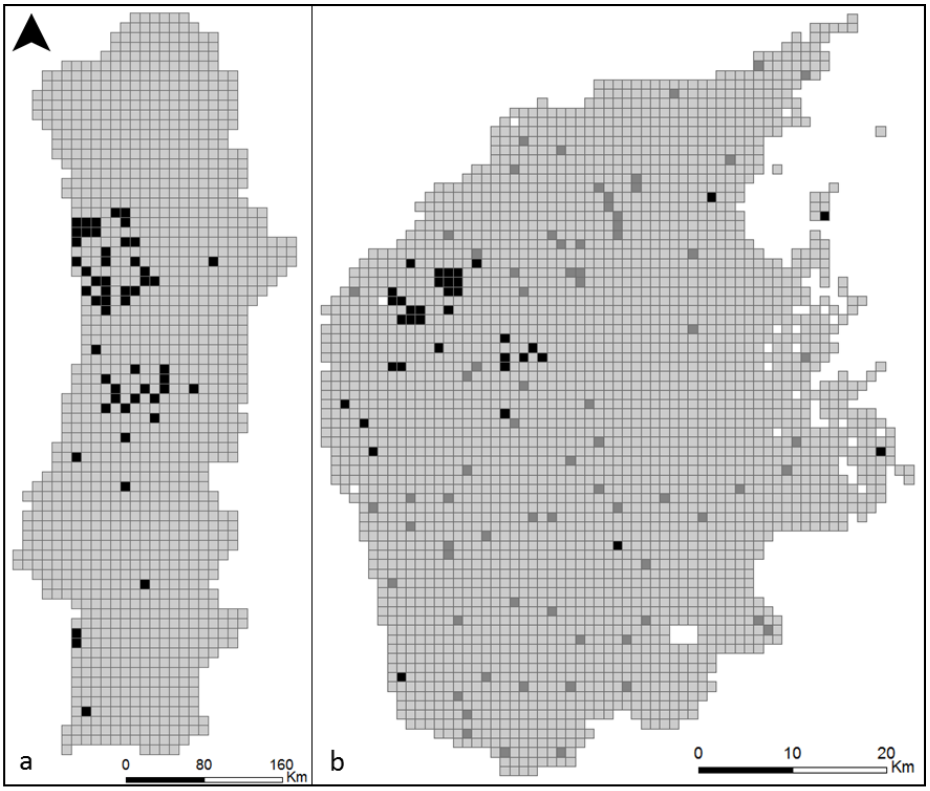

Figure III.3 - Known occurrences of *Hakea sericea* in Portugal and Galicia (a, 10x10km cell grid) and presence/absence records for the local scale study area in the Minho region (b, 1x1km cell). Cells with known occurrence are represented in black, and cells with confirmed absence are dark grey.

Sousa et al. (2004) reported, for the first time in Portugal, a fungal infection of *H. sericea* leafs, by *Pestalotiopsis funerea*. Considering the previously described use of another fungal species in biological control of *H. sericea* in South Africa, this result present a considerable interest for future biological control attempts in Portugal, a fact that is also discussed in the work of Sousa et al. (2004). In recent studies, Luís et al. (2014) used samples from Portuguese populations of *H. sericea* to show that extracts from the plant possess antimicrobial activity, particularly in preventing and reducing formation of biofilms of *Staphylococcus aureus*. Moreover, plant extracts, particularly from fruits, displayed selective toxicity over cells from a breast cancer cell line, indicating potential anticarcinogenic activity (Luís et al., 2014).

IIId. Biology and ecology

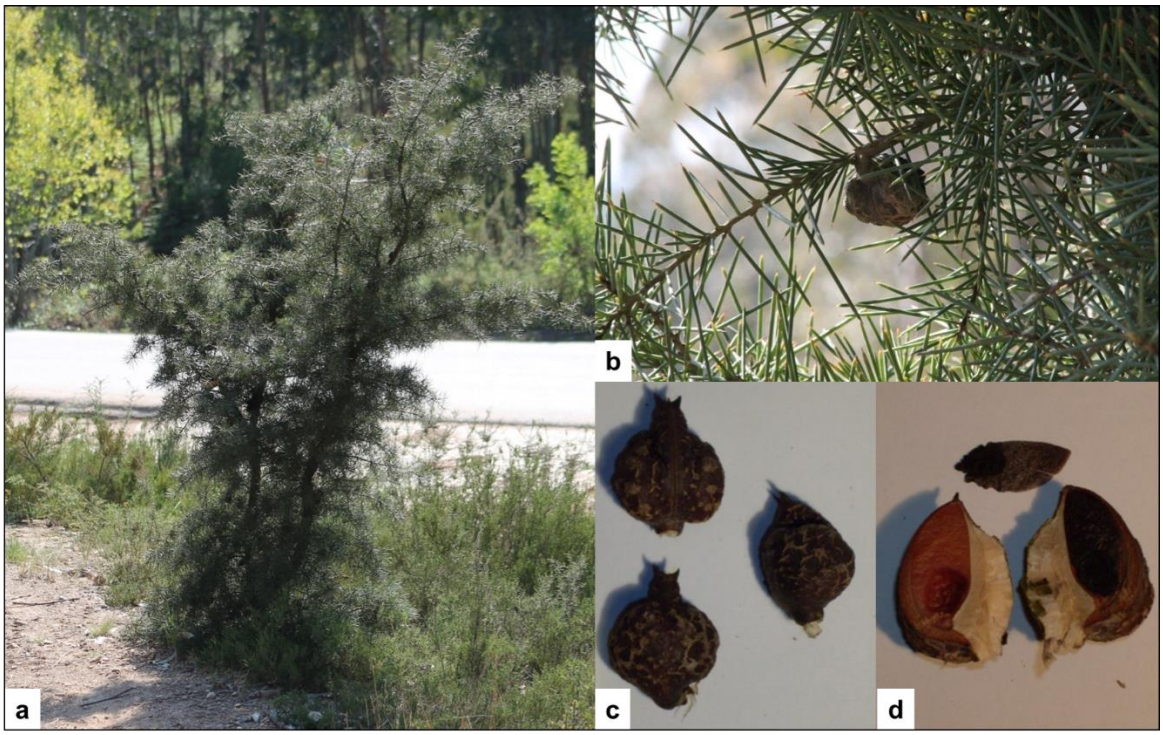

Figure III.4 - *Hakea sericea*: Single individual (a), detail of woody fruit attached to branch and of acicular leaves (b). Details of fruit, closed (c) and open, showing winged seeds (d). One of the seeds was removed for easier viewing.

Most *Proteaceae* species are able to develop proteoid roots (=cluster roots), secondary roots that form high-density clusters of determinate, lateral rootlets with root hairs, developed in response to low nutrient concentration (mainly of phosphorus), and that appear to play an important role in the absorption of nutrients, both by increasing the surface area and by exporting exudates to improve nutrient availability (Watt & Evans, 1999). For example, a response from *Hakea sericea* plants to phosphorous concentration in hydroponics was described by Sousa et al. (2007), with proteoid root development in low P (phosphorus) concentration but no development in high P concentration. Moreover, autoradiographs of roots incubated with ^{32}Pi showed a larger concentration in proteoid portions of the roots, compared to non-proteoid portions, again corroborating the idea that these structures play a role in nutrient absorption, with the authors considering the possibility that adaptive advantages may arise from this adaptations when invading Pi impoverished soils.

In a similarly nutrient related feature, seeds of many *Proteaceae* species, while not storing starch, may have a disproportionately high allocation of phosphorous and nitrogen, in comparison with relatively low carbon allocation (Myerscough et al., 2001). *H. sericea*, as well as other Australian *Proteaceae*, presents a higher phosphorous content in seeds than several *Proteaceae* species from South Africa (Mitchell & Allsopp, 1984), where *H. sericea* presents invasive behaviour.

In a growth experiment with South African and Australian *Proteaceae* (*Hakea sericea* among them) Stock et al. (1990) observed that N and P stored in cotyledons were enough to sustain prolonged seedling development, while reserves of cations Calcium, Potassium and Magnesium were not, having to "scavenge them from the growth medium" (the seeds were sowed on nutrient deficient sand). The authors stated that this difference in storage may be explained as adaptive to life in fire-prone environments – commonly there are rich deposits of cations in post-fire soil surface, but not very much available N or P, and therefore the reserves of N and P may work as a supplement, while Ca, K and Mg are obtained from the soil.

Adaptation to life in fire-prone environments may also be inferred from the fact that *H. sericea* fruits (Figure III.4 b, c, d) are what Groom & Lamont (1997) classify as "strongly serotinous", that is, the fruits remain closed for more than five years after fruit maturity. Indeed, serotiny refers in general to the prolonged retainment of seeds in the canopy (Lamont et al., 1991), and is usually interpreted as being a fire adaptation (Weston, 2007). In *H. sericea*, the seeds (Figure III.4 d) are retained in woody fruits that are, as in all the genus *Hakea*, follicles, derived from a single carpel with two ovules that develop into two winged seeds (Weston, 2007). Once mature, the fruit's thick woody walls offer protection to the seeds against heat (Brown & Whelan, 1999) and granivores (Groom & Lamont, 1997). This, together with a slow decrease in germination rates over time (Richardson et al., 1987; Lamont et al., 1991; Brown & Whelan, 1999), results in a large canopy-stored seed bank formed by seeds produced in a given year and viable seeds from previous years, which are released only after the death of the plant (Richardson et al., 1987). Therefore, an event such as a wildfire can result in the sudden release of a very large number of seeds.

IIIe. References

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